	L #	Hits	Search Text	DBs	Time Stamp
1	L1	74	hbx	USPAT; US-PGPUB	2002/03/11 17:50
2	L2	32	(hepatitis adj b adj virus or hbv) near4 inhibi? wrong truncation	USPAT; US-PGPUB	2002/03/11 18:08
3	L3	3	1 and 2	USPAT; US-PGPUB	2002/03/11 18:09
4	L4	0	1 near4 inhibit?	USPAT; US-PGPUB	2002/03/11 18:07
5	L5	0	1 near8 inhibit?	USPAT; US-PGPUB	2002/03/11 18:07
6	L6	254	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/03/11 18:18
7	L7	10	1 and 6	USPAT; US-PGPUB	2002/03/11 18:09
8	L8	3	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/03/11 18:19

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	74	hbx	USPAT; US-PGPUB	2002/03/11 17:50
2	L2	32	(hepatitis adj b adj virus or hbv) near4 inhibit? wrong truncation	USPAT; US-PGPUB	2002/03/11 18:08
3	L3	3	1 and 2	USPAT; US-PGPUB	2002/03/11 18:09
4	L4	0	1 near4 inhibit?	USPAT; US-PGPUB	2002/03/11 18:07
5	L5	0	1 near8 inhibit?	USPAT; US-PGPUB	2002/03/11 18:07
6	L6	254	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/03/11 18:08
7	L7	10	1 and 6	USPAT; US-PGPUB	2002/03/11 18:09

/

US-PAT-NO: 6071734
DOCUMENT-IDENTIFIER: US 6071734 A
TITLE: HBV polymerase, RNase H enzyme derived from HBV polymerase, processes for preparation and uses for screening antiviral agents thereof
DATE-ISSUED: June 6, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yoon; Sung-June	Seoul	N/A	N/A	KRX
Kim; Jong-Woo	Anyang-si	N/A	N/A	KRX
Huh; Yong	Kunpo-si	N/A	N/A	KRX
Rho; Hyune-Mo	Socho-ku	N/A	N/A	KRX
Jung; Gu-Hung	Seoul	N/A	N/A	KRX

US-CL-CURRENT: 435/199,435/252.3 ,435/254.11 ,435/320.1 ,435/325 ,435/410 ,435/69.7

ABSTRACT:
The present invention relates to hepatitis B virus (hereinafter it refers to HBV) polymerase containing a histidine tag, RNase H enzyme derived from HBV polymerase and processes for preparation thereof. More particularly, the present invention relates to recombinant HBV polymerase, its RNase H domain with enzyme activity, expression vectors producing the enzymes in E. coli and processes for preparing the HBV polymerase and the RNase H enzyme which can be easily purified due to their histidine tags. And the present invention relates to uses of the HBV polymerase and the RNase H enzyme for screening antiviral agents.

10 Claims, 17 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 15

DATE FILED: January 11, 1999

---------- KWIC ---------

BSPR:
HBV is a DNA virus, a member of the hepadnaviridae family, which has a spherical structure composed of nucleocapsid and core. HBV genome is a partially double stranded DNA of only 3.2 kb size, which is not a circular form. In detail, HBV genome is composed of four overlapped genes that are the polymerase (P) gene, the surface protein (HBsAg; S, pre-S1, pre-S2) gene, the core protein (HBcAg; pre-C, C) gene and X protein (**HBx**) gene. Among these genes, X protein gene encodes regulatory protein, and the other genes encode structural proteins of HBV. The polymerase gene occupies 80% of the total genome and encodes 94 KD-sized protein composed of 845 amino acids.

BSPR:
Particularly, the present invention provides methods for screening **inhibitors of the HBV** polymerase and the RNase H enzyme.

DEPR:
In order to select **HBV inhibitors working at the multiplication stage of HBV** by using the HBV polymerase,

DEPR:
In order to select **HBV inhibitors working at the multiplication stage of HBV** by using the RNase H domain of the HBV polymerase,

DEPU:
(d) the results of (c) stage is compared with those of comparative sample which does not contain a antiviral agent in the reaction mixture and used to calculate the **inhibitory effects of HBV** multiplcation.

DEPU:
(d) the results of (c) stage is compared with those of comparative sample which does not contain an antiviral agent in the reaction mixture and used to calculate the **inhibitory effects of HBV** multiplication.

US-PAT-NO: 6025341
DOCUMENT-IDENTIFIER: US 6025341 A
TITLE: Chimeric hepatitis B/hepatitis C virus vaccine
DATE-ISSUED: February 15, 2000
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Wands; Jack R.	Waban	MA	N/A	N/A
Tokushige; Katsutoshi	Boston	MA	N/A	N/A
Wakita; Takaji	Tokyo	N/A	N/A	JPX

US-CL-CURRENT: 514/44,530/350 ,536/23.1
ABSTRACT:
Nucleic acid molecule that comprise an incomplete hepatitis C and hepatitis B viral genome including specifically disclosed DNA sequences are disclosed. Pharmaceutical compositions that contain nucleic acid molecules comprising an incomplete hepatitis C and hepatitis B viral genome including a nucleotide sequence encoding a complete hepatitis C core protein and hepatitis B S gene protein operably linked to regulatory elements functional in human cells are disclosed. Methods of immunizing individuals susceptible to or infected by hepatitis B virus and/or hepatitis C virus comprising the step of administering such pharmaceutical compositions are disclosed.
60 Claims, 3 Drawing figures
Exemplary Claim Number: 1,4,31,34
Number of Drawing Sheets: 3

DATE FILED: May 12, 1997

---------- KWIC ---------

BSPR:
The HBV genome encodes for 4 open reading frames (ORF) that includes: 1) the S gene encoding for the envelope protein with 2 in-frame pre-S1 and pre-S2 polypeptides; 2) the polymerase ORF encoding for a reverse transcriptase protein that is responsible for reverse transcription of a 3.6 kb pregenomic RNA into DNA; 3) the core gene encoding for a protein that is assembled to complete the viral nucleocapsid; and 4) the **HBx** ORF encodes for a protein of unknown function. The pol gene encompasses 800% of the genome and overlaps with the other three ORFs. The core gene is preceded by an in-frame sequence that encodes for a signal peptide and following proteolytic cleavage gives rise to an antigenically distinct protein called the HBeAg. The **HBx** protein was found not to be essential for the viral life cycle in vitro (Blum, et al., J. Virol., 1992, 66, 123-127), but it appears to be necessary for the establishment of productive infection in vivo (Chen, et al., J. Virol., 1993, 67, 1218-1226). **HBx** can function as a transcriptional transactivator on a variety of cellular and viral genes and suggests that is may contribute to HCC development (Schek, et al., "The Hepadnaviral X Protein", In Molecular Biology of the Hepatitis B Virus, 1991, Vol.9, CRC Press, Boca Raton, Fla., pp.181-192).

ORPL:
Guidotti, L. et al., "Cytotoxic T. Lymphocytes **Inhibit Hepatitis B Virus** Gene Expression by a Noncytolytic Mechanism in Transgenic Mice", PNAS USA, 1994, 91, 3764-3768.

US-PAT-NO: 5985829
DOCUMENT-IDENTIFIER: US 5985829 A
TITLE: Screening assays for compounds that cause apoptosis and related compounds
DATE-ISSUED: November 16, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harris; Curtis C.	Bethesda	MD	N/A	N/A
Wang; Xin Wei	North Potomac	MD	N/A	N/A
Hoeijmakers; Jan H. J.	Zevenhuizen	N/A	N/A	NLX

US-CL-CURRENT: 514/12,514/13 ,514/14 ,514/15 ,530/324 ,530/326 ,530/328
ABSTRACT:
This invention relates to methods of screening for compounds capable of inducing apoptosis in certain tumor cells. The invention also relates to compounds identified by such methods. In addition, the invention relates to methods for the in vitro diagnosis of Xeroderma pigmentosum and compounds useful in these methods.
6 Claims, 13 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 9

DATE FILED: July 1, 1996

---------- KWIC ---------

DRPR:
FIG. 4. Peptides corresponding to helicase motif III of XPB and the C-terminus of p53 prevent XPB from binding to GST-p53. Four different synthetic peptides were preincubated with 2 .mu.g GST-p53WT for 30 minutes on ice before the addition of .sup.35 S-labeled, in vitro-translated XPB for 60 min at RT. Peptide #464 corresponds to residues 464-478 of XPB (lanes 2-4; 12, 120, and 596 nM), peptide #479 corresponds to residues 479-493 of XPB (lanes 5-7; 12, 116, and 578 nM), peptide #99 corresponds to residues 100-115 of **HBX** (lanes 8-9; 111 and 554 nM), and peptide #p53cp corresponds to residues 367-387 of p53 (lanes 11-12; 85 and 424 nM).

DEPR:
In order to demonstrate the binding of several mutant p53 proteins to NER proteins, equal amounts of GST-tagged p53 mutants, (135Y, 249S, 273H) were tested for binding to the in vitro-translated XPD, XPB, CSB and Rad3 proteins used above. The mutant p53 proteins were produced as described in Example 1 and were tested for binding to NER proteins in the same manner as was wild type p53. The results are shown in FIG. 1. All the mutants tested had similar or increased binding to the human proteins but decreased binding to yeast Rad3, as compared to GST-p53-WT (see FIG. 1A, comparing lane 3 with lanes 4, 5, and 6). While not wishing to be bound by theory, this opens the possibility that mutant p53 may exert a dominant negative effect by binding to and sequestering the

cellular targets of wild-type p53. The p53-135Y mutant, which has diminished binding to hepatitis B virus X protein and papillomavirus E6 protein, binds to XPD, XPB, Rad3 and CSB. **HBX** has also been shown to inhibit p53 binding to XPB presumably by binding to the same site of p53 that interacts with XPB, since **HBX** did not bind to XPB in vitro (see Wang, X. W., et al. (1994) Proc. Natl. Acad. Sci., USA91:2230-2234.)

ORPL:
Wang, X.W., et al. (1994) "Interaction with **hepatitis B virus X proteins inhibits** p53 transcriptional activity and p53 associated with ERCC3", Proceedings of the American Association For Cancer Research, Abstracts 35:585 (3486).

ORPL:
Wang, X.W., et al. (1994) "**Hepatitis B virus X protein inhibits** p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3", Proc. Natl. Acad. Sci. USA, 91:2230-2234.

US-PAT-NO: 5968781
DOCUMENT-IDENTIFIER: US 5968781 A
TITLE: HBV polymerase processes for preparation and uses for screening antiviral agents thereof
DATE-ISSUED: October 19, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Yoon; Sung-June	Seoul	N/A	N/A	KRX
Kim; Jong-Woo	Anyang-si	N/A	N/A	KRX
Huh; Yong	Kunpo-si	N/A	N/A	KRX
Rho; Hyune-Mo	Socho-ku	N/A	N/A	KRX
Jung; Gu-Hung	Seoul	N/A	N/A	KRX

US-CL-CURRENT: 435/69.7,435/194 ,435/252.33 ,435/320.1 ,536/23.2 ,536/23.4
ABSTRACT:
The present invention relates to hepatitis B virus (hereinafter it refers to HBV) polymerase containing a histidine tag, RNase H enzyme derived from HBV polymerase and processes for preparation thereof.

More particularly, the present invention relates to recombinant HBV polymerase, its RNase H domain with enzyme activity, expression vectors producing the enzymes in E. coli and processes for preparing the HBV polymerase and the RNase H enzyme which can be easily purified due to their histidine tags.

And the present invention relates to uses of the HBV polymerase and the RNase H enzyme for screening antiviral agents.
9 Claims, 17 Drawing figures
Exemplary Claim Number: 8
Number of Drawing Sheets: 15

DATE FILED: August 15, 1997

---------- KWIC ---------

BSPR:
HBV is a DNA virus, a member of the hepadnaviridae family, which has a spherical structure composed of nucleocapsid and core. HBV genome is a partially double stranded DNA of only 3.2 kb size, which is not a circular form. In detail, HBV genome is composed of four overlapped genes that are the polymerase (P) gene, the surface protein (HBsAg; S, pre-S1, pre-S2) gene, the core protein (HBcAg; pre-C, C) gene and X protein (**HBx**) gene. Among these genes, X protein gene encodes regulatory protein, and the other genes encode structural proteins of HBV. The polymerase gene occupies 80% of the total genome and encodes 94 KD-sized protein composed of 845 amino acids.

BSPR:
Particularly, the present invention provides methods for screening **inhibitors**

of the HBV polymerase and the RNase H enzyme.

DEPR:
In order to select **HBV inhibitors working at the multiplication stage of HBV** by using the HBV polymerase,

DEPR:
In order to select **HBV inhibitors working at the multiplication stage of HBV** by using the RNase H domain of the HBV polymerase,

DEPV:
(d) the results of (c) stage is compared with those of comparative sample which does not contain a antiviral agent in the reaction mixture and used to calculate the **inhibitory effects of HBV** multiplication.

DEPV:
(d) the results of (c) stage is compared with those of comparative sample which does not contain an antiviral agent in the reaction mixture and used to calculate the **inhibitory effects of HBV** multiplication.

US-PAT-NO: 5856459
DOCUMENT-IDENTIFIER: US 5856459 A
TITLE: Oligonucleotides specific for hepatitis B virus
DATE-ISSUED: January 5, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Frank; Bruce L.	Marlborough	MA	N/A	N/A
Roberts; Peter C.	Holliston	MA	N/A	N/A
Goodchild; John	Westborough	MA	N/A	N/A
Craig; J. Charles	Welwyn Garden	N/A	N/A	GBX
Mills; John S.	Welwyn Garden	N/A	N/A	GBX

US-CL-CURRENT: 536/24.5
ABSTRACT:
The present invention discloses synthetic oligonucleotides complementary to contiguous and noncontiguous regions of the HBV RNA. Also disclosed are methods and kits for **inhibiting the replication and expression of HBV,** and for treating HBV infections and associated conditions.
21 Claims, 17 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 11

DATE FILED: June 6, 1995

---------- KWIC ---------

ABPL:
The present invention discloses synthetic oligonucleotides complementary to contiguous and noncontiguous regions of the HBV RNA. Also disclosed are methods and kits for **inhibiting the replication and expression of HBV,** and for treating HBV infections and associated conditions.

BSPR:
Antisense oligonucleotides have been designed which **inhibit the expression and/or replication of HBV**. For example, antisense oligonucleotides directed against the cap site of HBV mRNA transcribed from the SPII promoter (Goodarzi et al. (1990) J. Gen. Virol. 71:3021-3025; Yao et al. (1994) Nat. Med. J. China 74:125), against the translational initiation site of the S gene (Yao et al. (1994) Nat. Med. J. China 74:125; Reinis et al. (1993) Folia Biologica (Praha) 39:262-269; Goodarzi et al. (1990) J. Gen. Virol. 71:3021-3025); against a portion of the core-pol mRNA encoding the terminal protein region of the viral polymerase (WO 94/24864; Blum et al. (1991) Lancet 337:1230), and against the HBV polyadenylation signal (Wu et al. (1992) J. Biol. Chem. 267:12436-12439) have been designed. In addition, phosphorothioate oligodeoxynucleotides prepared against the 5' region of the pre-S gene have been shown to **inhibit duck HBV** replication and gene expression in vivo (Offensperger et al. (1993) EMBO J. 12:1257-1262).

BSPR:
A need still remains for the development of oligonucleotides that are capable of **inhibiting the replication and expression of HBV** whose administration are accompanied by a good prognosis and low or no cellular toxicity.

BSPR:
It has been discovered that specific oligonucleotides complementary to particular contiguous and noncontiguous portions of pregenomic and messenger RNA encoding the precore, core, and polymerase proteins of **HBV can inhibit HBV** replication, packaging, and expression. This discovery has been exploited to provide synthetic oligonucleotides complementary to various contiguous and noncontiguous regions of HBV RNA.

BSPR:
Another aspect of the invention are kits for **inhibiting HBV** replication and/or infection in a cell. In preferred embodiments, the kits include at least one contiguous or noncontiguous oligonucleotide of the invention, or a combination thereof. In other preferred embodiments, at least two synthetic oligonucleotides of the invention are in the kit.

BSPR:
In yet another aspect of the invention, a therapeutic amount of a pharmaceutical composition containing HBV-specific synthetic oligonucleotides is administered to the cell in a method of **inhibiting HBV** replication. The HBV-specific oligonucleotides are the contiguous or noncontiguous oligonucleotides of the invention. In some preferred embodiments, the method includes administering at least one oligonucleotide, or at least two oligonucleotides, having a sequence set forth in the Sequence Listing as SEQ ID NO:1-31, 32-41, or 42-48, or a combination thereof.

DEPR:
HBV is a compact, enveloped DNA virus belonging to the Hepadnavirus family. It has a circular, partially single-stranded, partially double-stranded 3.2 kb genome which includes four overlapping genes: (1) the pre-S and S genes, which encode the various envelope or surface antigens (HBsAg); (2) the preC and C gene, which encodes the antigens HBcAg and HBeAg; (3) the P gene, which encodes the viral polymerase; and (4) the X gene, which encodes **HBx,** the transactivating protein. Full-length clones of many hepadnaviruses have been obtained and their nucleotide sequences obtained. (see, e.g., Raney et al. in Molecular Biology of the Hepatitis B Virus (McLachlan, ed.) CRC Press, Boston, Mass., (1991) pp. 1-38). Replication occurs in hepatocytes and involves converting the single stranded-region of the HBV genome to double-stranded circular DNA, generating the covalently closed circular (CCC) DNA. Transcription of this DNA by the host RNA polymerase generates an RNA template of plus stranded polarity, the pregenomic RNA, which serves as a template for the translation of viral proteins, and is also encapsidated into virus cores. In the virus cores, the RNA serves as a template for reverse transcription, generating a DNA minus strand. The viral polymerase then produces a DNA plus strand using an oligomer of viral RNA as a primer. The newly synthesized double-stranded DNA in the viral core is assembled with the viral envelope proteins, generating a newly infectious viral particle.

DEPR:
Antisense oligonucleotide technology provides a novel approach to the **inhibition of HBV** expression, and hence, to the treatment or prevention of acute and chronic hepatitis and hepatocellular carcinoma (see generally, Agrawal (1992) Trends Biotech. 10:152; and Crooke (Proc. Am. Ass. Cancer Res. Ann. Meeting (1995) 36:655). By binding to the complementary nucleic acid sequence, antisense oligonucleotides are able to inhibit splicing and translation of RNA, and replication of genomic RNA. In this way, antisense oligonucleotides are able to inhibit protein expression.

DEPR:
The activity of antisense oligonucleotides was also studied in a viral assay in HepG2.2.15 cells, which have been stably transfected with plasmids carrying whole HBV genomes (Sells et al. (1987) Proc. Nat. Acad. Sci. 84:1005-1009; Sureau et al. (1986) Cell 47:37-47). While a number of assays for **HBV inhibitors** based on the HepG2 2.2.15 cell line have been reported (Jansen et al. (1993) Antimicrob. Agent. Chemother. 37:441-447; Korba et al. (1992) Antiviral Res. 19:55-70), these involve the detection of HBV DNA by means of dot blot or PCR, tests which do not provide data concerning the precise source of the measured DNA. A more definitive test is Southern hybridization, which provides data concerning the character of the detected DNA in addition to quantitation. This assay has been described previously for the screening of anti-HBV compounds on HepG2.2.15 cells (Doong et al. (1991) Proc. Nat. Acad. Sci. (USA) 88:8495-8499). In view of the many potential sources of HBV DNA from transfected cells, this assay allows for a more meaningful interpretation of results than the other methods mentioned. When HBV6 (SEQ. ID NO:45) was titrated, significant inhibition was found (FIG. 11). Inhibition was also found to be mediated by the stem-loop bridging oligonucleotide, HBV67 (SEQ. ID NO:37) (FIG. 12).

DEPR:
The results of this experiment demonstrate that the **HBV-specific oligonucleotides of the invention have inhibitory** activity.

DEPR:
The synthetic antisense oligonucleotides of the invention may be in the form of a therapeutic composition or formulation useful in **inhibiting HBV** replication in a cell, and in treating hepatitis B infections and associated conditions in an animal, such as acute and chronic hepatitis and hepatocellular carcinoma. They may be used as part of a pharmaceutical composition when combined with a physiologically and/or pharmaceutically acceptable carrier. The characteristics of the carrier will depend on the route of administration. Such a composition may contain, in addition to the synthetic oligonucleotide and carrier, diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The pharmaceutical composition of the invention may also contain other active factors and/or agents which enhance **inhibition of HBV** expression. For example, combinations of synthetic oligonucleotides, each of which is directed to different regions of the HBV nucleic acid, may be used in the pharmaceutical compositions of the invention. The pharmaceutical composition of the invention may further contain other chemotherapeutic drugs for the treatment of hepatocellular carcinoma. Such additional factors and/or agents may be included in the pharmaceutical

composition to produce a synergistic effect with the synthetic oligonucleotide of the invention, or to minimize side-effects caused by the synthetic oligonucleotide of the invention. Conversely, the synthetic oligonucleotide of the invention may be included in formulations of a particular anti-HBV or anti-cancer factor and/or agent to minimize side effects of the anti-HBV factor and/or agent.

DEPR:
The oligonucleotides of the invention may also be a part of kits for **inhibiting HBV** replication and infection in a cell. Such a kit includes a synthetic oligonucleotide specific for HBV nucleic acid, such as those described herein. For example, the kit may include at least one of the synthetic contiguous oligonucleotides of the invention, such as, but not limited to, those having SEQ ID NO: 1-31 and 42-48. These oligonucleotides may have modified backbones, such as those described above, and may be RNA/DNA hybrids containing, for example, at least one 2'-O-methyl. The kit of the invention may optionally include buffers, cell or tissue preparation reagents, cell or tissue preparation tools, vials, and the like.

ORPL:
Korba et al. Antisense oligonucleotides are effective **inhibitors of hepatitis B virus** replication in vitro Antiviral Research vol. 28 225-242, 1995.

US-PAT-NO: 5853716
DOCUMENT-IDENTIFIER: US 5853716 A
TITLE: Genetically engineered chimeric viruses for the treatment of diseases associated with viral transactivators
DATE-ISSUED: December 29, 1998
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Tattersall; Peter J.	Guilford	CT	N/A	N/A
Cotmore; Susan F.	Guilford	CT	N/A	N/A

US-CL-CURRENT: 424/93.2,424/93.6 ,435/357 ,435/372.3 ,536/24.1
ABSTRACT:
The present invention relates to chimeric viruses, the replication of which is regulated by a transactivation signal produced by diseased host cells. The chimeric viruses of the invention can infect both normal and diseased host cells. However, the chimeric virus replicates efficiently in and kills diseased host cells that produce the transactivation signal. The use of such chimeric viruses to treat infectious diseases and cancers are described. A particularly useful embodiment involves the modification of a murine parvovirus that infects human T cells to generate a chimeric parvovirus that is cytocidal to human T cells that express HIV-tat. The chimeric parvovirus can be used to treat HIV-infection.
12 Claims, 16 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 12

DATE FILED: July 25, 1996

---------- KWIC ---------

DEPR:
The viruses which can be used for purposes of the invention include, but are not limited, to parvoviruses, retrovirus, adenovirus, adeno-associated virus, herpes virus, vaccinia virus, etc. The choice of virus will depend, in part, on the nature of the disease to be treated, taking into account the tropism of the virus (natural or engineered) for the host cells which are targets for the disease. The transactivator-responsive element, or TRE, that is engineered into the viral genome in order to construct the chimeric virus will also depend upon the disease to be treated. For example, retroviral LTRs which bind HIV-tat can be used as TREs for the construction of chimeric viruses useful for treating HIV-infection; HBEnI and HBEnII elements of HBV which are responsive to the **HBX** protein can be used as TREs for engineering chimeric viruses to treat HBV-infection; Tax responsive elements of HTLV can be used as TREs to engineer chimeric viruses responsive to Tax to treat HTLV-induced leukemias; Myc responsive elements can be used as TREs to engineer chimeric viruses to treat cancers which overexpress Myc, i.e. Burkitt's lymphoma, small cell lung carcinomas, glial blastomas; ICP4 responsive elements of HSV can be used as TREs to engineer chimeric viruses to treat HSV; the HOX11 responsive element

can be used as TREs for engineering chimeric viruses to treat T cell acute leukemias.

DEPR:
In other embodiments, elements of the early promoter region of MVM can be replaced with TRE sequences derived from the regulatory regions of the hepatitis B virus (HBV). Such chimeric viruses could be used to treat HBV-infection. The hepatitis B virus **HBX** protein is a transactivator of transcription which is required for viral infection and has been implicated in virus-mediated liver oncogenesis (Zahm et al., 1988, Oncogene 3:169-177). **HBX** transactivates both Enhancer I and II of the HBV genome (Spandau and Lee, 1988, J Virol. 62:427-434). The dependence of viral infection upon **HBX** expression may provide its use as an antagonist to interfere with and suppress HBV infection.

DEPR:
MVM plasmids can be modified to generate the chimeric constructs, pMVM-HBEnI and pMVM-HBEnII. The pMVM-HBEnI plasmid would contain the **HBX** responsive region, Enhancer region I, which extends from nucleotide 950 to 1150 of the 3.2-kb HBV genome (Spandau and Lee, 1988, J Virol. 62:427-434). The pMVM-HBEnII plasmid contains the **HBX** responsive region, Enhancer region II, which extends from nucleotide 1646 to 1715. These inserts replace the analogous sequences present in the MVM P4 promoter region (FIG. 2).

DEPR:
Additional cytocidal-antagonist viruses may be constructed in which nucleotide sequences that encode a gene product or transcript that **inhibits HBV** replication can be engineered into a non-essential or complementable region of the chimeric parvoviral genome. For example, the HBV surface antigen, core antigen and viral DNA polymerase of HBV can be targeted to disrupt HBV replication using the methods including, but not limited to: antisense RNAs, triple helix, ribozyme approaches, and dominant negative mutants as outlined above.

DEPR:
In another embodiment of the invention, non-cytocidal-antagonist vectors can be designed to **inhibit HBV** replication while allowing the host cell to survive. The pMVM-HBEnI and pMVM-HBEnII virus can be engineered to encode antagonist **HBX** sequences, including but not limited to **HBX** antisense, ribozymes that cleave **HBX** mRNA, and short transcripts designed to form triple helix structures with the **HBX** regulatory region, thereby preventing transcription of **HBX**.

US-PAT-NO: 5834186
DOCUMENT-IDENTIFIER: US 5834186 A
TITLE: Regulatable RNA molecule
DATE-ISSUED: November 10, 1998
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
George; Shaji T.	New York	NY	N/A	N/A
Shih; Andy	New York	NY	N/A	N/A
Bockman; Jeffrey Michael	New York	NY	N/A	N/A

US-CL-CURRENT: 435/6,435/91.1 ,435/91.31 ,536/23.1 ,536/23.2 ,536/24.5
ABSTRACT:
Regulatable RNA molecules such as regulatable ribozymes, nucleic acids encoding such regulatable ribozymes, and methods of making and using such regulatable ribozymes are disclosed. Regulatable ribozymes comprise a ligand-binding RNA sequence and a ribozyme sequence capable of cleaving a separate targeted RNA sequence, wherein upon binding of the ligand to the ligand-binding RNA sequence, the activity of the ribozyme sequence against the targeted RNA sequence is altered. The ligand may be either an inorganic or an organic molecule and may be a co-drug which can be administered to specifically regulate the ribozyme activity. Regulatable RNA molecules other than ribozymes are also disclosed, such as regulatable mRNA molecules which comprise a ligand-binding RNA sequence separate from the coding sequence, wherein upon binding of a ligand to the ligand-binding RNA sequence, translation of the regulatable mRNA is altered.
16 Claims, 12 Drawing figures
Exemplary Claim Number: 1,9
Number of Drawing Sheets: 11

DATE FILED: June 2, 1995

---------- KWIC ---------

DEPR:
Another example is the situation in hepatocellular carcinoma associated with hepatitis B infection. Evidence suggests that the virus genome, which is integrated into the host cell DNA in liver cells, is responsible for the conversion of that cell into a tumor cell. This can be by expression of viral genes, the surface antigen or a trans-activating factor **hbx,** or a consequence of the integration itself. In the latter case, inactivation of viral gene expression by ribozyme cleavage of the surface antigen or **hbx** would have no effect. What is desirable is ribozyme cleavage of an RNA essential to cell survival so that the cell dies. However, the delivery process is often non-specific, so that the ribozyme could be delivered to cells not harboring the virus, which would be killed also. If the ribozyme is active only in the presence of the surface antigen protein or **hbx** protein, then only those cells with virus would be killed.

DEPC:
Inhibition of cleavage of HBV substrate by Cibacron Blue dye.

US-PAT-NO: 5741679
DOCUMENT-IDENTIFIER: US 5741679 A
TITLE: Regulatable nucleic acid therapeutic and methods of use thereof
DATE-ISSUED: April 21, 1998
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
George; Shaji T.	New York	NY	N/A	N/A
Shih; Andy	New York	NY	N/A	N/A
Bockman; Jeffrey Michael	New York	NY	N/A	N/A

US-CL-CURRENT: 435/91.31
ABSTRACT:
Regulatable RNA molecules such as regulatable ribozymes, nucleic acids encoding such regulatable ribozymes, and methods of making and using such regulatable ribozymes are disclosed. Regulatable ribozymes comprise a ligand-binding RNA sequence and a ribozyme sequence capable of cleaving a separate targeted RNA sequence, wherein upon binding of the ligand to the ligand-binding RNA sequence, the activity of the ribozyme sequence against the targeted RNA sequence is altered. The ligand may be either an inorganic or an organic molecule and may be a co-drug which can be administered to specifically regulate the ribozyme activity. Regulatable RNA molecules other than ribozymes are also disclosed, such as regulatable mRNA molecules which comprise a ligand-binding RNA sequence separate from the coding sequence, wherein upon binding of a ligand to the ligand-binding RNA sequence, translation of the regulatable mRNA is altered.
28 Claims, 12 Drawing figures
Exemplary Claim Number: 15
Number of Drawing Sheets: 11

DATE FILED: September 16, 1994

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DEPR:
Another example is the situation in hepatocellular carcinoma associated with hepatitis B infection. Evidence suggests that the virus genome, which is integrated into the host cell DNA in liver cells, is responsible for the conversion of that cell into a tumor cell. This can be by expression of viral genes, the surface antigen or a trans-activating factor **hbx,** or a consequence of the integration itself. In the latter case, inactivation of viral gene expression by ribozyme cleavage of the surface antigen or **hbx** would have no effect. What is desirable is ribozyme cleavage of an RNA essential to cell survival so that the cell dies. However, the delivery process is often non-specific, so that the ribozyme could be delivered to cells not harboring the virus, which would be killed also. If the ribozyme is active only in the presence of the surface antigen protein or **hbx** protein, then only those cells with virus would be killed.

DEPC:
Inhibition of Cleavage of HBV Substrate by Cibacron Blue Dye

US-PAT-NO: 5610050
DOCUMENT-IDENTIFIER: US 5610050 A
TITLE: Methods of preventing viral replication
DATE-ISSUED: March 11, 1997
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Blum; Hubert E.	Zurich	N/A	N/A	CHX
Liang; Tsanyang	Brookline	MA	N/A	N/A
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US-CL-CURRENT: 435/238,435/320.1 ,536/23.1 ,536/23.72 ,536/24.5
ABSTRACT:
The invention relates to methods and compositions for inhibition of viral replication. In particular, termination of replication of hepatitis B virus is achieved by introducing into a target cell an antisense oligonucleotide having a sequence substantially complementary to an mRNA which is in turn complementary to a portion of the minus strand of a hepatitis viral genome, which portion encoding solely part or all of the terminal protein region of the viral polymerase.
26 Claims, 23 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 13

DATE FILED: April 23, 1993

---------- KWIC ---------

BSPR:
Synthetic antisense oligonucleotides have been used as inhibitors of viral gene expression. Smith et al., Proc. Natl. Acad. Sci. USA, 2787-2791 (1986), report antiviral activity of an oligo(nucleoside methylphosphonate) complementary to the splice junction of herpes simplex virus type I immediate early pre-mRNAs 4 and 5. See also: Agris et al., Inhibition of vesicular stomatitis virus protein synthesis and infection by methylphosphonates, Biochem. 25, 6268-6275 (1986); Zamecnik et al., Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide, Proc. Natl. Acad. Sci. USA 75:280-284 (1978); and Zamecnik et al., Inhibition of replication and expression of human T-cell lymphotropic virus type III in cultured cells by exogenous synthetic oligonucleotides complementary to viral RNA, Proc. Natl. Acad. Sci. USA 83, 4143-4146 (1986). Goodarzi et al., J. Gen. Virol. 71:3021-3025 (1990), report **inhibition of expression of the gene for hepatitis B virus** surface antigen by antisense oligodeoxynucleotides directed at the cap site of mRNA and regions of the translational initiation site of the HBsAg gene.

BSPR:
The invention relates to antisense oligonucleotides, preferably antisense

oligodeoxynucleotides, as antiviral agents against HBV; pharmaceutical compositions providing such antiviral oligonucleotides; and methods for their use in **inhibiting HBV**. Antisense oligonucleotide compositions complementary to a region of RNA encoded by the HBV polymerase gene completely block viral transcription, antigen production, and replication. Preferred are antiviral oligonucleotides substantially complementary to RNA encoding a region of the terminal protein domain of the HBV polymerase protein. Preferred is such an antiviral oligonucleotide comprising at least about 12 nucleotides corresponding to a sequence of nucleotides from about n.t.s. 2850 to about n.t.s. 2794, more preferably from about n.t.s. 2833 to about n.t.s. 2794, of HBV subtype adw2, numbering according to the sequence published in Blum et al., Persistence of Hepatitis B Viral DNA After Serological Recovery from Hepatitis B Virus Infection, Hepatology 14, No. 1:56-63 (1991). The sequence of the (+) strand from n.t.s. 2357 to n.t.s. 3172 is as shown in SEQ ID NO. 49. Such antiviral oligonucleotides can be provided to the target cell either exogenously as an antisense DNA or RNA, or by insertion of a sense DNA sequence into an expression vector capable of producing the antisense oligonucleotides endogenously within the target cell.

DRPR:
FIGS. 5A and 5B: **Inhibition of viral antigen synthesis by HBV**-specific antisense oligodeoxynucleotide. Detection of HBsAg (5A) and HBeAg (5B) in cell culture medium after transfection of human hepatoma cells (HuH 7) with HBV-DNA alone (.oval-solid. control), HBV-DNA plus an antisense oligodeoxynucleotide (........box-solid........ ATC-40), or HBV-DNA plus a sense oligodeoxynucleotide (-------.tangle-solidup.------- GAT-40). Analyses were completed with commercially available radioimmunoassays (Centocor, Malvern, Pa. and Abbott, Chicago, Ill., USA).

DRPR:
FIG. 6: Map of the HBV genome showing the coding organization of the four major open reading frames (the pre-S and S gene which code for the HBsAg; the C gene, which codes for HBcAg and HBeAg; the P gene which codes for the HBV DNA polymerase; and the X gene, which codes for the transactivating X protein, **HBX**), as well as the 3.5 kb pregenomic RNA and the 2.1 kb subgenomic RNA species.

DRPR:
FIG. 11: Effect of oligodeoxynucleotides on HBV DNA replication. Left hand side represents Southern blot analysis of HBV DNA derived from cell lysates 5 days 5 following co-transfection. Co-transfection with the antisense oligodeoxynucleotide ATC 30 but not the sense construct resulted in complete **inhibition of HBV** DNA replication. Analysis of HBV virions secreted into the medium revealed a similar effect of the various oligonucleotide constructs as shown on the right hand side of the figure.

DEPR:
Four major open reading frames (ORFs) encoded by the HBV minus strand have been identified and characterized: 1) the pre-S and S gene, which code for the HBsAg and several other less well characterized gene products; 2) the C gene, which codes for HBcAg and HBeAg; 3) the P gene, which codes for the viral DNA polymerase; and 4) the X gene, which codes for the transactivating X protein,

HBx, seen more frequently in patients with hepatocellular carcinoma. Within the HBV polymerase gene is a region encoding the terminal protein domain of the polymerase protein, which encompasses the initial approximately 25% of the amino terminal end of the polymerase ORF, followed by a spacer region, the DNA polymerase and the RNAase H regions. The polymerase ORF of HBV subtype adw2 extends from about n.t.s. 2357 to about n.t.s. 1625, with the region coding for the terminal protein domain located from about n.t.s. 2357 to about n.t.s. 3172 (numbering as published in Blum et al., Persistence of Hepatitis B Viral DNA After Serological Recovery from Hepatitis B Virus Infection, Hepatology 14, No. 1:56-63 (1991)). The corresponding sequences for other strains or subtypes of HBV can be determined by those of skill in the art by aligning homologous sequences, e.g., by using sequence data available in GenBank.RTM.. For example, it was determined in this manner that the polymerase ORF of the HBV Hep strain is found from about n.t.s. 2357 to 1620. The nucleotide sequences of various hepatitis virus strains can be found in Okamoto et al., J. Gen. Virol. 69:2575-2583 (1988) and through GenBank. The teachings of these references is hereby incorporated by reference. The complete nucleotide sequence of HBV mutant 5-15, which exhibits closest homology with published sequences for HBsAg subtype adw2, is reported in Blum et al., Persistence of Hepatitis B Viral DNA After Serological Recovery from Hepatitis B Virus Infection, Hepatology 14, No. 1: 56-63, at 58 (1991), the teaching of which is incorporated herein by reference. See FIG. 7, which sets forth a map of the HBV viral genome, showing the organizational structure of the four major ORFs and the pregenomic and subgenomic RNA species.

DEPR:
It has been determined that the antiviral oligomers of the invention which are complementary to part or all of a region of HBV RNA (mRNA/genomic RNA) encoding the HBV polymerase, preferably a region encoding a portion of the polymerase protein terminal protein domain, can totally block viral replication. "Antisense" describes the interaction of oligonucleotides (which can be RNA, DNA, or a combination thereof) with cellular nucleic acid targets in a sequence-specific manner, more particularly, the interaction of oligonucleotides (having the sequence of the HBV minus strand DNA) with their complementary **HBV genomic RNA or mRNA, to inhibit HBV** viral replication. The interaction of antisense oligonucleotides with their receptor sequences results from hybridization interactions. Antisense oligonucleotides inhibit viral replication, including inhibition of the production of the protein product, in this case, the HBV polymerase protein. The therapeutic applications of antisense oligonucleotides in general are described, e.g., in the following review articles: Le Doan et al., Antisense Oligonucleotides as Potential Antiviral and Anticancer Agents, Bull. Cancer 76:849-852 (1989); Dolnick, B. J., Antisense Agents in Pharmacology, Biochem. Pharmacol. 40:671-675 (1990); Crooke, Annu., Rev. Pharmacol. Toxicol. 32, 329-76 (1992).

DEPR:
The invention relates to a composition of matter consisting essentially of an antiviral antisense oligonucleotide of the invention, preferably an oligodeoxynucleotide, consisting of a sequence of at least 11 nucleotides (preferably at least 12, more preferably at least 15, and most preferably at least 18) substantially complementary to an RNA (e.g., messenger RNA or genomic RNA) which is complementary to a portion of the minus strand of a hepatitis

viral genome, which portion encodes solely part or all of the terminal protein domain of the HBV polymerase. Because the antisense oligonucleotides are substantially complementary to their respective HBV RNA sequences, these complementary sequences can hybridize under physiological conditions, with resultant inhibition of viral replication. Antisense oligonucleotides of the invention have been shown to be capable of completely **inhibiting HBV** replication. Accordingly, the invention also relates to methods of **inhibiting HBV** replication in cells containing HBV, including methods of preventing HBV infection in an animal (e.g., a human or other mammal, or a bird) exposed to HBV, and methods of treating an animal infected with HBV. The invention also relates to pharmaceutical compositions for use in preventing HBV infection in an animal exposed to HBV, or treating an animal infected with HBV. Preferred are such pharmaceutical compositions formulated for parenteral administration. Such pharmaceutical compositions will contain an effective antiviral amount of an oligonucleotide of the invention and a pharmaceutically acceptable carrier.

DEPR:
While the use of a defective polymerase protein to inhibit replication is discussed generally, it is recognized that the defective protein is specific for its own virus. That is, a mutation in the hepatitis B virus polymerase gene will produce a defective protein which **inhibits or terminates hepatitis B virus** replication.

DEPR:
It has been found that 25-, 30-, and 40-mer antisense oligonucleotides substantially complementary to RNA encoded by the HBV polymerase gene, preferably the terminal protein region of that gene, completely block HBV antigen production and replication, whereas the complementary sense oligonucleotides of similar lengths have little or no effect. Moreover, 30-mer antisense oligodeoxynucleotides from conserved regions of the HBV core gene (n.t.s. 1865-1894) and the X gene (n.t.s. 1428-1457), did not reduce viral protein (HBsAg and HBcAg) or **inhibit HBV** replication. Antisense constructs directed to this same region but of 16 or 20 nucleotides in length exhibited antiviral activity, but to a lesser degree. Accordingly, the invention relates to antisense oligonucleotides of at least about 12 nucleotides in length, and preferably up to about 80 nucleotides in length, more preferably about 15 to 40 nucleotides in length, still more preferably about 15 to 30 nucleotides in length, and most preferably about 20 to 30 nucleotides in length.

DEPR:
As used herein, "consisting essentially of" has its usual meaning, i.e., that one or more compositions of matter of the invention may be used together, either in admixture or combined in a single molecule, with other materials that do not alter the essential nature of the invention. For example, while the antisense oligonucleotide sequences of the invention are essential to the invention, it is contemplated that they may be used in admixture or in chemical combination with one or more other materials, including other oligonucleotides antisense to HBV RNA, materials that increase the biological stability of the oligonucleotides, or materials that increase their ability to selectively penetrate their hepatocyte target cells and reach and hybridize with their target RNA. Furthermore, the term "oligonucleotide" includes derivatives thereof, such as backbone modifications, e.g., phosphorothioate derivatives,

employed to stabilize the oligonucleotides. All such modifications are contemplated equivalents of the antisense oligonucleotides of the invention. The following discussion provides examples of the kinds of modifications that may be employed, but those of skill in the art will readily recognize others. For example, the antisense oligonucleotides may be provided in stabilized form, e.g., with phosphotriester linkages, or by blocking against exonuclease attack with methylphosphonodiester linkages, with 3' deoxythymidine, as a phenylisourea derivative, or by linking other molecules such as aminoacridine or polylysine to the 3' end of the oligonucleotide. See e.g., Anticancer Research 10:1169-1182, at 1171-2 (1990), the teaching of which is incorporated herein by reference. For antisense oligonucleotides supplied exogenously, increased selectivity for hepatocytes may be achieved by linking antisense oligonucleotides of the invention to natural ligands such as ASOR (asialoorosomucoid) or to synthetic ligands that will bind to the hepatic asialoglycoprotein (ASGP) receptor. See e.g., Biochemistry 29, No. 43 (1990), Spiess, "The Asialoglycoprotein Receptor: A Model for Endocytic Transport Receptors". See also Wu and Wu, J. Biol. Chem. 267, No. 18:12436-12439 (1992), reporting **inhibition of HBV** viral gene expression and replication in HepG2 cells by a 21-mer oligonucleotide complementary to the HBV polyadenylation signal. The oligomer was complexed to a (poly)L-lysine-asialoorosomucoid conjugate that targets the asialoglycoprotein receptor of hepatocytes. In another embodiment, ribozymes may be targeted by linking to an oligonucleotide of the invention. See e.g., Von Weiszaecker F., Blum H. E., Wands J. R., Three ribozymes transcribed from a single DNA template efficiently cleave hepatitis B virus pregenomic RNA, Biochem. Biophys. Res. Commun. 189:743-748 (1992). The teaching of the foregoing references is incorporated herein by reference.

DEPR:
The 25-mer antisense oligonucleotide sequence is contained within the ATC-40 antisense oligonucleotide which corresponds to HBV adw2 2794-2833 (described in Example II supra) and which also completely inhibits HBsAg accumulation. (Numbering herein of HBV subtype adw2 is according to Blum et al., Hepatology Vol. 14(No.1):56-63 (1991); see FIG. 1 at 58.) The 25-mer antisense oligonucleotide also **inhibits the replication of duck hepatitis B virus** in duck hepatocytes. Sequence complementarity studies indicate that only 12 out of the 25 nucleotides in this oligo are present as the complementary nucleotide in the duck hepatitis B virus genome. See Table 6 infra. The complementary nucleotides are present as a sequence of 11 nucleotides-ATTTTGCGGGT (SEQ ID NO: 38), corresponding to nucleotides 2815-2825 of the genome of HBV subtype adw2, and a 12th complementary nucleotide, T at nucleotide 2813. The conservation of this particular sequence of 11 or 12 nucleotides between the two viruses suggests that this sequence is important for viral viability. Although the conserved sequence is entirely contained within both the 25-mer antisense, WO 10042, and ATC-40, only four of the conserved nucleotides are represented in the 23-mer antisense oligonucleotide. Moreover, the inactive 18-mer included none of the conserved sequence. This may explain the decreased activity of the 23-mer and the inability of the 18-mer antisense oligonucleotide to turn off HBsAg expression.

DEPR:
Example I describes a naturally-occurring replication defective HBV mutant

found in the uninvolved liver of an individual with HCC. Fine molecular analysis of this variant HBV revealed an A to C mutation at position 2798 in the 5' region of the polymerase gene, resulting in a threonine to proline change in the terminal protein region of the viral polymerase. This amino acid change rendered the HBV non-replicative, due to an inability to package pregenomic RNA into the core particles. Thus, molecular characterization of this naturally occurring HBV mutant suggested a potential region of the viral mRNA that may be susceptible to attack by the action of antisense oligodeoxynucleotides. In this context, we explored the effects of oligodeoxynucleotide length, as well as concentration, on adw HTD HBV DNA gene expression and replication using a transient co-transfection system with HUH-7 cells. It was striking that 40, 30 and 25-mer antisense oligonucleotides in close proximity to or spanning the naturally occurring point mutation in the 5' region of the polymerase gene completely **inhibited HBV** gene expression and replication. Shorter constructs of 20 and 16 oligodeoxynucleotides were substantially less effective. Sense constructs had no **inhibitory effect as long as the optimal HBV** DNA target to oligonucleotide ratio of 1:5 (wt/wt) was maintained. Indeed, using this transient transfection system, the concentration of oligonucleotides was shown to be very important in assessing their anti-viral effects. For example, increasing concentrations of sense oligonucleotides (FIGS. 8A-8C) resulted in inhibition of HBsAg synthesis and secretion into the culture medium, particularly at a DNA target to oligodeoxynucleotide ratio of 1:10. Increasing the concentration of the sense oligodeoxynucleotide even further to a ratio of 1:20 resulted in complete inhibition of HBsAg synthesis. We interpret these findings to indicate that either the transfection efficiency diminishes substantially with increasing DNA concentration as previously observed by others (Chen and Okayama, supra) or the oligodeoxynucleotides at high concentrations are toxic to the cells. However, there appears to be little, if any, effect on HUH-7 cell viability as measured by Trypan Blue exclusion at the concentrations of oligodeoxynucleotides employed (data not shown). More importantly, we have identified a region of the polymerase mRNA, i.e., the terminal protein domain, that is particularly susceptible to the anti-viral effects of antisense oligodeoxynucleotides, since oligodeoxynucleotides derived from other highly conserved regions of the viral genome, namely, the core and X gene, had no such effect. Surprisingly, the region of mRNA highly susceptible to antisense attack is not limited to sequences encompassing mRNA corresponding to the site mutation at n.t.s. 2798 of the polymerase gene, but extends to a broader region of the terminal protein domain of the HBV polymerase gene. Furthermore, the magnitude of the antiviral effects exhibited by antisense oligonucleotides derived from the terminal protein domain of the HBV polymerase gene was noteworthy since there was 100% inhibition of viral gene expression and replication. A recent study of antisense oligodeoxynucleotides targeted via the asialogycoprotein receptor in HepG2 cells (2.2.1.5.) stably transfected with **HBV (Wu and Wu, Specific inhibition of hepatitis B virus** gene expression in vitro by targeted antisense oligodeoxynucleotides. J. Biol. Chem. 267:12436-12439 (1992)) revealed a maximal 80% reduction in HBsAg synthesis and HBV replication. In that study, a 21-mer oligodeoxynucleotide directed against the HBV polyadenylation signal was used as the antisense DNA. Other investigators have achieved antiviral effects varying between 50-80% specific inhibition of human immunodeficiency virus infection (HIV) in vitro using 21-mer antisense oligodeoxy-nucleotides spanning various regions of the viral genome (Agrawal et al., Proc. Natl. Acad. Sci.

USA 86:7790-7794 (1989); Goodchild et al., Proc. Natl. Acad. Sci. USA 85:5507-5511 (1988)). In both of these studies, phosphorothioate derivatives were employed to render the oligodeoxynucleotides less susceptible to nuclease digestion. In the present investigation, we have not altered the native phosphodiester bonds of the oligodeoxynucleotide sequence to prolong the half-life and stability of the antisense constructs. It is likely, therefore, that the observed antiviral effects on intracellular HBV replication may be obtained at considerably lower concentrations of oligodeoxynucleotides if their stability can be improved without affecting binding affinity to target mRNA sequences.

DEPC:
Inhibition of Hepatitis B Virus by Antisense Oligodeoxynucleotide 40met

DEPC:
Inhibition of Hepatitis B Virus by Additional Antisense Oligodeoxynucleotides

CLPR:
1. A method for **inhibiting replication of hepatitis B virus** in a cultured cell, comprising introducing into said cell an antiviral antisense oligonucleotide the sequence of which consists of 12 to 40 contiguous nucleotides of (a) ATC-40: ATA TGG TGA CCC GCA AAA TGA TGC GCT ACG TGT GGG TTC C (SEQ ID NO: 11), or (b) the RNA equivalent of ATC-40, whereby **hepatitis B virus replication is inhibited**.

ORPL:
Blum et al., **Inhibition of hepatitis B virus** by antisense oligodeoxynucleotides, The Lancet 337:1230, 1991.

ORPL:
Offensperger, W., et al., "In Vivo **Inhibiton of Duck Hepatitis B Virus** Replication and Gene Expression By Phosphorothioate Modified Antisense Oligodeoxynucleotides", 1993, EMBO J., 12(3):1257-62.

US-PAT-NO: 5532124
DOCUMENT-IDENTIFIER: US 5532124 A
TITLE: Genetically engineered bacteria to identify and produce medically important agents
DATE-ISSUED: July 2, 1996
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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Grafstrom; Robert H.	Lansdowne	PA	N/A	N/A

US-CL-CURRENT: 435/5,435/184 ,435/23 ,435/244 ,435/252.3 ,435/34 ,435/6 ,435/68.1 ,435/69.1 ,435/69.2 ,435/974
ABSTRACT:
Microorganisms modified such that their growth in selective media is dependent upon the inhibition of a medically important target function are provided and utilized in methods for the screening of potential medically important compounds.
11 Claims, 6 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

DATE FILED: October 6, 1993

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BSPR:
Similarly, it is critical that a rapid screening assay for **inhibitors of HBV** be developed. HBV-is associated with chronic liver disease, including chronic hepatitis, cirrhosis and hepatocellular carcinoma. Infection with HBV can lead to one, or a combination, of the following outcomes: inapparent infection followed by seroconversion, acute hepatitis followed by recovery or death, and chronic infection. Chronically infected individuals possess HBV genetic information in their hepatocytes and often experience persistent viremia with an absence of neutralizing antibodies. These individuals may experience no frank symptoms and may carry the virus in their livers, and possibly elsewhere, for a period of years.

BSPR:
Methods for the identification of drugs and selection of microorganisms that produce **inhibitors of HIV protease, HBV** x kinase, HSV .alpha.4 protein, and HBV pregenome packaging; mediators of high blood pressure and mediators of inflammation are also provided.

DEPR:
In a preferred embodiment of the present invention the x kinase (**hbx**) can used as a target since it serves a vital viral function. HBV is a small incompletely double stranded DNA virus with a distinct liver tropism and a complicated life cycle. Expression of the viral genome is modulated by liver

specific enhancers and it codes for at least four gene products (core, pol, S and x), which are produced from overlapping genes. All hepadnaviruses appear to use a RNA replication intermediate as a template for their minus DNA strand synthesis and have been shown to induce a virus specific reverse transcriptase. HBV is believed to encode only two distinct enzymatic activities: the virus polymerase, which is a reverse transcriptase, and a transcriptional activator with protein kinase activity. These are the products of the pol and x genes, respectively.

DEPR:
Inhibitors of the **hbx** kinase will be detected by co-cultivation with TJU-hbx:tet.sup.x. Agents that inhibit **hbx** will be recognized by their ability to enhance the growth of the bacteria in tetracycline media. Since this is a positive growth assay wherein positives cause growth, not death, of the bacteria, false positives that nonspecifically kill E. coli will not be a complication. Agents that appear promising in this assay can than be promoted to further rigorous analysis in other conventional tissue culture tests. Optimum assay conditions will be determined by one skilled in the art. Methods for the co-cultivation of this strain with candidate compounds and mixtures will be developed. This should be straightforward to one skilled in the art by testing of compounds for their ability to enhance growth of this strain in tetracycline. Crude compounds derived from plant extracts, chemical mixtures and soil samples will be co-cultivated with the strain. Any mixture observed to consistently elevate the growth of this strain, and not affect **hbx** minus strains, will be examined. For example, crude mixtures will be fractionated by whatever separation methods are appropriate for the source, and retested by the co-cultivation assay. Fractions exhibiting positive growth effects will be further resolved by chromatography and monitored for activity in the bacterial growth assay. When the compound is believed to be sufficiently enriched and pure, it will be tested directly for its ability to: inhibit the **hbx** kinase in the conventional kinase assay; and, **inhibit the growth of HBV** in tissue culture. This bacteriological assay will allow one to screen many compounds from a range of sources that would not be possible to test in other systems.

DEPR:
Although TJU-hbx:tet.sup.x is unable to thrive on agar plates containing tetracycline, under conditions of **hbx** gene product induction, it is believed that rare viable variants will emerge at a frequency of approximately 1 in 10,000. These mutants will be isolated and are expected to belong to the following classes: those that have an altered tetracycline metabolism; those that have deleted the x gene sequence or have lost the ability to express the x gene; those that have altered the Tet-x protein or its expression level; and, those that produce an inhibitor of the **hbx** kinase.

DEPR:
It will be necessary to be able to easily distinguish one class of mutants from the other to decide which mutants are the most useful as antiviral compounds. Straightforward genetic and biochemical strategies for categorizing mutants as they arise are included in the present invention. The classification strategy, briefly, involves determining if the mutation lies within the host chromosome or plasmids. If the mutation lies within the host chromosome, it will be examined further for the possibility that they produce an inhibitor of **hbx**.

For example, material from such mutants will be tested for the ability to enhance the growth of either TJU-hbx:tet.sup.x under induction of **hbx** conditions or tet-x (lacking **hbx**) strains in tetracycline media. If such material enhances only the growth of TJU-hbx:tet.sup.x strains, but not tet-x, it will be considered very likely that the mutant bacteria is producing an inhibitor of **hbx**. Such mutants will be pursued for characterization and isolation by both the bacteriological and biochemical assays used by those skilled in the art. Such assays will include, for example, kinase assays.

DEPR:
Mutants that possess altered Tet metabolism, x gene lesions, or altered Tet-x protein or expression, will also be fully characterized because they will reveal useful genetic or structural information about tetracycline metabolism or **hbx** function.

DEPR:
The **hbx** gene, derived from woodchuck hepatitis virus, was cloned into a plasmid. The PBR322 tetracycline gene has been modified to contain the 7 amino acid sequence designed to be recognized by the **hbx** gene product. The modified tet gene is called tet-x and confers tetracycline resistance to sensitive strains. Therefore, the insertion of these amino acids into the tet gene in frame does not eliminate Tet function. The modified protein elaborated by this gene is called Tet-x. The tet-x and **hbx** genes have been molecularly cloned into E. coli strain JM109. The doubly transformed strain is called TJU-hbx:tet.sup.x. Since the basis of these experiments requires that Tet-x be modified by **hbx,** it will be important to determine if Tet-x is a substrate for **hbx** kination. Preliminary experiments indicate that the Tet-x can undergo kination at serine residues, as anticipated by design. Direct identification of kination will serve two functions. First, it will confirm that **hbx** is, as expected, functional-as isolated from bacteria as a protein kinase. Second, it will provide biochemical evidence that the tet-x protein behaves as expected. Further, **hbx** and kinase assays will also be performed. Tet-x will be prepared by in vitro transcription and translation reactions. Specificity of the reactions will be monitored by use of wildtype tet protein as substrate. Since wildtype tet does not contain the amino acid sequences predicted to be kination sites for **hbx,** wild type tet should not be an efficient substrate.

DEPR:
Having produced the relevant strains and demonstrated that Tet-x is, indeed, subject to kination by **hbx,** it is expected that a bacteria strain such as TJU-hbx:tet.sup.x, which expresses both **hbx** and tet-x will unable to grow in tet media when the **hbx** gene is induced. Further, it is expected that growth in tetracycline media will be restored by repression of **hbx**. It has been shown that E. coli expressing both **hbx** as well as the wildtype tet protein are as viable and grow vigorously in tetracycline media as do strains that lack **hbx**. In these strains **hbx** is expressed to as much as 0.5% of total protein mass. It is clear that expression of **hbx,** per se, at these levels is not growth inhibitory. Thus, any growth sensitivity of TJU-hbx:tet.sup.x tetracycline media under conditions where the **hbx** gene is induced will be considered evidence that the bacteria can not grow because **hbx** is modifying Tet-x. FIGS. 4 through 6 demonstrate that TJU-hbx:tet.sup.x does not grow in tetracycline

containing media when the **hbx** gene is induced. Control experiments, using bacterial strain Oligo 3, which contains a modified tet-x gene but no x gene, show bacterial growth under IPTG induction.

DEPR:
These experiments will be conducted over a range of tetracycline concentrations and levels of **hbx** induction. The level of **hbx** induction and phosphorylation status of Tet-x will be biochemically determined by kinase assays and SDS gel electrophoresis. Control kinase experiments will be carried out on wild type Tet in control strains. It is expected that Tet-x, as isolated from bacteria, will be phosphorylated in response to **hbx** induction and the cells will be unable to grow in tetracycline. This will be determined directly by phospho-amino acid analysis. Similarly, it is expected that wild type Tet will remain unphosphorylated or incompletely phosphorylated, as isolated from bacteria induced for **hbx,** and that these bacteria will grow vigorously in tetracycline media. Preliminary results suggest that these expectations are correct. It is therefore believed that it is possible to construct tet-x/hbx expresser strains whose growth depends, at least phenotypically, upon repression of the **hbx**.

DEPR:
The present invention includes a bacterial strain whose growth in tetracycline media requires that the packaging function of **HBV pol and core proteins be inhibited**. Bacteria have been constructed that possess a tetracycline resistance mRNA gene product that contains the 64 nucleotide packaging site recognized by HBV pol and core gene products. This E. coli strain has been designated TJU-etet-pol/core. It is believed that when this bacteria expresses an appropriate amount of HBV pol and core gene product, the tet mRNA will become complexed with pol and core protein and be unable to become translated into tet protein. Bacteria will be unable to grow in tetracycline media when core and pol are functional. Preliminary results using TJU-etet-pol/core suggest that this strain can not grow in tetracycline containing media when core and pol proteins are made. Therefore, **inhibitors of HBV** packaging can be screened for by the co-cultivation methods outlined above: compounds are tested for anti-packaging activity by testing their ability to enhance the growth of this bacteria in tetracycline media. Moreover, mutant bacteria can be selected for by plating the bacteria in tetracycline media and selecting rare isolates. These rare isolates may include mutant bacteria that produce **inhibitors of HBV** packaging. These mutants can be distinguished from the other kinds of mutants that may occur by the methods and logic outlined above. Briefly, they are first tested for the presence of plasmids with the original tet and core and pol genotypes. If they have the original core and tet and pol genotypes, they will be tested further for the possibility that they are producing inhibitors of packaging by testing bacterial extracts for their ability to **inhibit of HBV** by conventional mammalian tissue culture assays.

DEPL:
Determination if tet-x Can Serve as Substrate for **hbx**

DEPL:
Examination of Growth of TJU-hbx:tet.sup.x in Tetracycline Media and Dependence Upon the Inhibition of **hbx**

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	74	hbx	USPAT; US-PGPUB	2002/03/11 17:50
2	L2	32	(hepatitis adj b adj virus or hbv) near4 inhibit?	USPAT; US-PGPUB	2002/03/11 18:08
3	L3	3	1 and 2	USPAT; US-PGPUB	2002/03/11 18:09
4	L4	0	1 near4 inhibit?	USPAT; US-PGPUB	2002/03/11 18:07
5	L5	0	1 near8 inhibit?	USPAT; US-PGPUB	2002/03/11 18:07
6	L6	254	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/03/11 18:18
7	L7	10	1 and 6	USPAT; US-PGPUB	2002/03/11 18:09
8	L8	3	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/03/11 18:19

US-PAT-NO: 5985829
DOCUMENT-IDENTIFIER: US 5985829 A
TITLE: Screening assays for compounds that cause apoptosis and related compounds
DATE-ISSUED: November 16, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Harris; Curtis C.	Bethesda	MD	N/A	N/A
Wang; Xin Wei	North Potomac	MD	N/A	N/A
Hoeijmakers; Jan H. J.	Zevenhuizen	N/A	N/A	NLX

US-CL-CURRENT: 514/12,514/13 ,514/14 ,514/15 ,530/324 ,530/326 ,530/328
ABSTRACT:
This invention relates to methods of screening for compounds capable of inducing apoptosis in certain tumor cells. The invention also relates to compounds identified by such methods. In addition, the invention relates to methods for the in vitro diagnosis of Xeroderma pigmentosum and compounds useful in these methods.
6 Claims, 13 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 9

DATE FILED: July 1, 1996

---------- KWIC ---------

DEPR:
In order to demonstrate the binding of several mutant p53 proteins to NER proteins, equal amounts of GST-tagged p53 mutants, (135Y, 249S, 273H) were tested for binding to the in vitro-translated XPD, XPB, CSB and Rad3 proteins used above. The mutant p53 proteins were produced as described in Example 1 and were tested for binding to NER proteins in the same manner as was wild type p53. The results are shown in FIG. 1. All the mutants tested had similar or increased binding to the human proteins but decreased binding to yeast Rad3, as compared to GST-p53-WT (see FIG. 1A, comparing lane 3 with lanes 4, 5, and 6). While not wishing to be bound by theory, this opens the possibility that mutant p53 may exert a dominant negative effect by binding to and sequestering the cellular targets of wild-type p53. The p53-135Y mutant, which has diminished binding to hepatitis B virus X protein and papillomavirus E6 protein, binds to XPD, XPB, Rad3 and CSB. **HBX has also been shown to inhibit** p53 binding to XPB presumably by binding to the same site of p53 that interacts with XPB, since HBX did not bind to XPB in vitro (see Wang, X. W., et al. (1994) Proc. Natl. Acad. Sci., USA91:2230-2234.)

US-PAT-NO: 5872206
DOCUMENT-IDENTIFIER: US 5872206 A
TITLE: Compositions and methods for interfering wtih hepatitis B virus infection
DATE-ISSUED: February 16, 1999
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liang; Tsanyang Jake	Brookline	MA	N/A	N/A
Huang; Jiakang	Cambridge	MA	N/A	N/A

US-CL-CURRENT: 530/300,530/324 ,530/326 ,530/350 ,530/412
ABSTRACT:
The invention provides for compositions and methods for interfering with Hepatitis B viral infection that are based on the interaction of Hepatitis B virus X protein with a novel proteasome subunit.
4 Claims, 10 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 8

DATE FILED: October 6, 1994

---------- KWIC ---------

BSPR:
HBV is a partially double-stranded circular genome enclosed in a core structure surrounded by a lipid bilayer envelope containing Hepatitis B surface protein. HBV has a unique fourth open reading frame coding for a 16 kDa protein known as HBX. HBX appears to possess multiple functions. It activates a variety of viral and cellular promoters in diverse cell types (Colgrove et al., J. Virol. 63:4109-4026, 1989; Seto et al., Nature 344:72-74, 1990; Maguire et al., Science 252:842-844, 1991; Cross et al., Proc. Natl. Acad. Sci. USA 90:8078-8082, 1993), and therefore is a transactivator. Although the X protein does not bind to DNA directly, it activates transcription when it is targeted to a promoter by fusion to a heterologous DNA binding domain (Seto et al., supra; Maguire et al., supra; Cross et al., supra; Unger et al., The Eur. Mol. Biol. Org. J. 9:1889-1895, 1990). The protein has also been shown to function through AP-1 and AP-2 (Seto et al., supra) and to interact directly with members of CREB/ATF transcription factor family (Maguire et al., supra). Furthermore, a "Kunitz domain," characteristic of kunitz-type serine protease **inhibitors, is present in HBX,** and mutation of this consensus sequence inactivates the transactivation function of HBX (Takada et al., Jpn. J. Cancer Res. 81:1191-1194, 1990). In a transgenic mouse model, HBX has been shown to induce development of hepatocellular carcinoma (Kim et al., Nature 315:317-320, 1991). HBX also has been shown to play an essential role in HBV infection in vivo (Chen et al., J. Virol. 67:1218-1226, 1993; Zoulim et al., J. Virol. 68:2026-2030, 1994).

DEPR:

Structural and functional mapping of HBX have defined two domains that are crucial for the transactivation function of HBX (Takada et al., supra; Arii et al., Oncogene 7:397-403, 1992; Runkel et al., Virology 197:529-536, 1993). These two domains appear to overlap with the putative "Kunitz-type" domain of protease **inhibitor that are present in both HBX** and WHVX. Several key residues in these two domains were mutated and studied with respect to the transactivation function of HBX and interaction between HBX and XAPC7 in the yeast two-hybrid system. The glycine and cysteine residues were mutated in both domains (FIG. 4); several other conserved residues were also mutated. In FIG. 4, amino acid sequences of HBX and WHVX around the putative Kunitz Domains (underlined) are shown. Amino acid numbers of the HBX protein are shown at the top. Site-directed mutations (shown at the bottom of the amino acid residues) were introduced and are numbered as MT1 to 10 sequentially: MT1 with Cys to Ser mutation at aa #61, MT2 with Gly to Ala mutation at aa #67, MT3 with Pro to Ala at aa #68, MT4 with Cys to Ser at aa #69, MT5 with Trp to Arg at aa #120, MT6 with Phe to Tyr at aa #132, MT7 with Gly to Val at aa #136, MT8 with Cys to Ser at aa #137, MT9 with Arg to Gln at aa #138, MT10 with His to Asp at aa #139. An additional mutant, MT11, was generated by replacement of aa residues 137 to 141 (Cys-Arg-His-Lys-Leu) with Val-Met sequence. The HBXRsr mutant has been described previously.

US-PAT-NO: 5532124
DOCUMENT-IDENTIFIER: US 5532124 A
TITLE: Genetically engineered bacteria to identify and produce medically important agents
DATE-ISSUED: July 2, 1996
INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Block; Timothy M.	Doylestown	PA	N/A	N/A
Grafstrom; Robert H.	Lansdowne	PA	N/A	N/A

US-CL-CURRENT: 435/5,435/184 ,435/23 ,435/244 ,435/252.3 ,435/34 ,435/6 ,435/68.1 ,435/69.1 ,435/69.2 ,435/974

ABSTRACT:
Microorganisms modified such that their growth in selective media is dependent upon the inhibition of a medically important target function are provided and utilized in methods for the screening of potential medically important compounds.
11 Claims, 6 Drawing figures
Exemplary Claim Number: 1
Number of Drawing Sheets: 6

DATE FILED: October 6, 1993

---------- KWIC ---------

DEPR:
Inhibitors of the hbx kinase will be detected by co-cultivation with TJU-hbx:tet.sup.x. Agents that **inhibit hbx** will be recognized by their ability to enhance the growth of the bacteria in tetracycline media. Since this is a positive growth assay wherein positives cause growth, not death, of the bacteria, false positives that nonspecifically kill E. coli will not be a complication. Agents that appear promising in this assay can than be promoted to further rigorous analysis in other conventional tissue culture tests. Optimum assay conditions will be determined by one skilled in the art. Methods for the co-cultivation of this strain with candidate compounds and mixtures will be developed. This should be straightforward to one skilled in the art by testing of compounds for their ability to enhance growth of this strain in tetracycline. Crude compounds derived from plant extracts, chemical mixtures and soil samples will be co-cultivated with the strain. Any mixture observed to consistently elevate the growth of this strain, and not affect hbx minus strains, will be examined. For example, crude mixtures will be fractionated by whatever separation methods are appropriate for the source, and retested by the co-cultivation assay. Fractions exhibiting positive growth effects will be further resolved by chromatography and monitored for activity in the bacterial growth assay. When the compound is believed to be sufficiently enriched and pure, it will be tested directly for its ability to: **inhibit the hbx** kinase in the conventional kinase assay; and, inhibit the growth of HBV in tissue culture. This bacteriological assay will allow one to screen many compounds

from a range of sources that would not be possible to test in other systems.

DEPR:
Although TJU-hbx:tet.sup.x is unable to thrive on agar plates containing tetracycline, under conditions of hbx gene product induction, it is believed that rare viable variants will emerge at a frequency of approximately 1 in 10,000. These mutants will be isolated and are expected to belong to the following classes: those that have an altered tetracycline metabolism; those that have deleted the x gene sequence or have lost the ability to express the x gene; those that have altered the Tet-x protein or its expression level; and, those that produce an **inhibitor of the hbx** kinase.

DEPR:
It will be necessary to be able to easily distinguish one class of mutants from the other to decide which mutants are the most useful as antiviral compounds. Straightforward genetic and biochemical strategies for categorizing mutants as they arise are included in the present invention. The classification strategy, briefly, involves determining if the mutation lies within the host chromosome or plasmids. If the mutation lies within the host chromosome, it will be examined further for the possibility that they produce an **inhibitor of hbx**. For example, material from such mutants will be tested for the ability to enhance the growth of either TJU-hbx:tet.sup.x under induction of hbx conditions or tet-x (lacking hbx) strains in tetracycline media. If such material enhances only the growth of TJU-hbx:tet.sup.x strains, but not tet-x, it will be considered very likely that the mutant bacteria is producing an **inhibitor of hbx**. Such mutants will be pursued for characterization and isolation by both the bacteriological and biochemical assays used by those skilled in the art. Such assays will include, for example, kinase assays.

DEPL:
Examination of Growth of TJU-hbx:tet.sup.x in Tetracycline Media and Dependence Upon the **Inhibition of hbx**